Ovine Epididymitis: *Brucella ovis*

Contagious Epididymitis

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Importance

Brucella ovis, a bacterium that circulates in sheep, is an economically important cause of epididymitis and reduced fertility in rams. Although this organism is also associated occasionally with abortions and increased perinatal mortality, its primary effect is on the male. Rams can become persistently infected, and transmit *B. ovis* to other males. Ewes are relatively resistant to infection, and if they become infected, clear the organism in a short time. Farmed red deer in New Zealand are the only other animals confirmed to be infected by *B. ovis* in nature. However, experimental infections have been reported in goats, bighorn sheep, white-tailed deer and pregnant cows, and there are a few reports of antibodies in goats. Cervids, goats and bighorn sheep sometimes developed epididymitis. Unlike most species of *Brucella*, *B. ovis* does not seem to infect humans.

Etiology

Ovine epididymitis is caused by *Brucella ovis*, a Gram-negative coccobacillus in the family Brucellaceae (class Alphaproteobacteria). Other species of *Brucella*, especially *B. melitensis*, can also infect sheep. Information about *B. melitensis* is available in the factsheets at http://www.cfsph.iastate.edu/DiseaseInfo/factsheets.htm.

Note on taxonomy: At one time, the genus *Brucella* was reclassified into a single species, *B. melitensis*, based on the genetic and immunological evidence that all members of this genus are closely related. Under this system, the various species of *Brucella* were considered to be biovars. This proposal was controversial, and it has fallen out of favor for practical reasons.

Species Affected

B. ovis can circulate in sheep, the primary hosts for this organism, and farmed red deer (*Cervus elaphus*). Experimental infections have been established in goats, cattle, Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) and white-tailed deer (*Odocoileus virginianus*). There are reports of antibodies in goats from South America, Europe (Bulgaria) and South Africa; however, naturally-acquired infections have not been confirmed in this species by bacteriology, as of 2018. Bighorn sheep in the U.S. are reported to be seropositive in rare cases. One attempt to infect mouflon (*Ovis musimon*) by intraconjunctival injection did not result in clinical signs or bacteriological evidence of infection, and antibody titers became undetectable within a few months. Experimental infections have been established in gerbils and rats by artificial routes such as intratesticular inoculation; however, most rodents and rabbits did not seem to be very susceptible to infection.

Zoonotic potential

B. ovis is not known to infect humans.

Geographic Distribution

B. ovis has been reported from Australia, New Zealand, North and South America, parts of Africa and Asia, and some countries in Europe. While some experts have suggested that this organism probably occurs in most sheep-raising regions of the world, some countries report never having seen it. Other countries have only recently documented its presence.

Transmission

Rams often become persistently infected with *B. ovis*, with many animals shedding this organism in semen for 2-4 years or longer. Shedding can occur with or without clinical signs, and it can be intermittent. Rams can also excrete this organism in urine. In many cases, *B. ovis* is thought to be transmitted from ram to ram via ewes, which can act as mechanical vectors even if they do not become infected. Such ewes have been reported to carry this organism in the vagina for at least 2 months. *B. ovis* can also be transmitted directly between rams, and from rams to red deer stags. Ram-to-ram transmission is poorly understood; however, experiments indicate that this organism can enter the body via the prepuce, conjunctiva, and oral, nasal and rectal mucosae. Proposed routes include preputial licking or other forms of oral transmission, and sexual

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transmission when rams mount each other. Ewes occasionally become infected, and may shed this organism in vaginal discharges and milk. However, these infections seem to be transient and rarely span more than one pregnancy. Lambs could be infected if they nurse from an infected dam, but congenitally infected lambs seem to be rare. There is one unpublished report that shearing handpieces contaminated with semen can transmit *B. ovis*.

Red deer can also be infected by venereal transmission, direct contact between infected stags, and experimentally by the conjunctival, nasal and rectal routes. Like rams, infected stags shed *B. ovis* in semen and urine; however, most animals eliminate the infection within a year. Goats have ben infected by inoculating organisms onto the nasal, conjunctival or preputial mucosa (as well as by intratesticular injection), while white-tailed deer and bighorn sheep were infected via the conjunctiva. Experimentally infected white-tailed deer, bighorn sheep and some goats shed *B. ovis* in semen, and deer transmitted it to uninfected stags kept in the same enclosure. This organism was also found in aborted fetal tissues from bighorn sheep. One of two experimentally infected, pregnant cows excreted *B. ovis* in milk.

Contamination of pastures does not seem to be an important method of transmission. Sheep did not become infected when they grazed fields that had recently been occupied by infected animals, or when they were kept in enclosures next to infected animals. Other species of *Brucella* have been reported to survive in the environment for periods ranging from less than a day to > 8 months, depending on factors such as temperature, humidity, exposure to sunlight and the presence of organic matter. Survival is longer when the temperature is low. There is no evidence that arthropods play any role in the epidemiology of brucellosis; however, some species of *Brucella* have been detected in ticks and other blood-sucking arthropods, *B. abortus* has been transmitted to guinea pigs via tick bites in the laboratory, and transovarial transmission of *B. melitensis* was reported in ticks.

Disinfection

Brucella spp. are readily killed by most commonly available disinfectants including hypochlorite solutions, 70% ethanol, isopropanol, iodophors, phenolic disinfectants, formaldehyde, glutaraldehyde and xylene. A 1% solution of citric acid was reported to be less effective. One study reported that xylene and calcium cyanamide decontaminated liquid manure after 2-4 weeks; however, some sources recommend storing such treated manure for much longer. Brucellae are inactivated fairly quickly by acid pH < 3.5. They can also be destroyed by moist heat of 121°C (250°F) for at least 15 minutes, dry heat of 320-338°F (160-170°C) for at least 1 hour, gamma irradiation and pasteurization. Boiling for 10 minutes is usually effective for liquids.

Incubation Period

In experimentally infected rams, clinically detectable lesions became apparent 3-17 weeks after inoculation. Changes in semen quality have been found as soon as one week after inoculation.

Clinical Signs

B. ovis can cause epididymitis, orchitis and impaired fertility in rams. Poor quality semen with increased numbers of white blood cells may initially be the only sign; sperm motility and concentration may be decreased, and individual sperm are often abnormal. Palpable lesions can be found in the epididymis and scrotum of an estimated 30-50% of animals. The lesions can be unilateral or bilateral, although unilateral lesions are reported to be more common. Epididymitis results in the enlargement of the epididymis, especially the tail, and pain or unusual discomfort may be apparent in some animals on palpation. Nodules or other abnormalities may also be detected, and the testis sometimes atrophies in chronic cases. Palpable lesions are permanent in most rams. *B. ovis* can also cause abortions, stillbirths and increased perinatal mortality, with some infected ewes giving birth to weak or small lambs. However, such reproductive losses are reported to be uncommon in the field. Systemic signs are rare in adult ewes and rams.

Similar signs and lesions, including epididymitis and poor semen quality, have been reported in red deer stags and experimentally infected male goats, bighorn sheep and white-tailed deer. Only a minority of infected red deer stags seems to have palpable lesions. Abortions and increased perinatal mortality have not been documented in red deer hinds, but abortions were seen in experimentally infected bighorn sheep. No clinical signs or lesions were detected in two experimentally infected, pregnant cows.

Post Mortem Lesions

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Gross lesions are mainly found in the epididymis, tunica vaginalis and testis of rams. One or both epididymes may be enlarged to varying degrees, with the tail of the epididymis affected more often than the head or body. Spermatoceles containing partially inspissated spermatic fluid may be detected in some animals. The tunica vaginalis is often thickened and fibrous, and extensive adhesions may be present. Fibrous atrophy can sometimes occur in the testis. Secondary microscopic lesions may be found in parts of the reproductive tract without gross lesions, including the ampulla ductus deferens and seminal vesicles. Similar lesions have been reported in red deer stags and experimentally infected bighorn sheep and white-tailed deer.

The typical lesions in the placenta are yellow-grey plaques, which often coalesce, in the intercotyledonary areas. The cotyledons may be necrotic. Gelatinous edema has been seen in the chorioallantois in severe cases. Mild to moderate pneumonic changes have been reported in the fetus.

Diagnostic Tests

Microscopic examination of semen, vaginal swabs, milk or tissue smears for *B. ovis*, using modified Ziehl-Neelsen (Stamp) staining, can be useful for a presumptive diagnosis. Brucellae are not truly acid-fast, but they are resistant to decolorization by weak acids and stain red. They appear as coccobacilli or short rods, usually arranged singly but sometimes in pairs or small groups. *B. ovis* appears identical to *Brucella melitensis* and also resembles some other organisms, including *Chlamydia abortus* and *Coxiella burnetii*. If available, immunostaining may aid identification. Definitive diagnosis requires culture and/or the detection of nucleic acids by PCR or other genetic techniques.

B. ovis may be isolated from semen samples in rams, vaginal swabs and milk in ewes, and the placenta or fetus (stomach contents and lung) after an abortion. Suspect rams can also be tested by collecting a vaginal sample from a *B. ovis*-free ewe after mating. Repeated sampling of semen may be necessary, as this organism can be shed intermittently. At necropsy, the preferred tissues to collect in rams include the epididymis, seminal vesicles, ampullae and inguinal lymph nodes. The uterus, iliac and supramammary lymph nodes can be sampled in ewes. Sampling additional lymph nodes (e.g., cranial, scapular, pre-femoral and testicular) and the spleen may increase the likelihood of detecting *B. ovis* in both sexes. *B. ovis* can be cultured on a variety of nonselective media, or on selective media such as Thayer-Martin or CITA medium. (Farrell's medium is often used to isolate other species of *Brucella*, but it inhibits *B. ovis*.) Enrichment techniques can also be employed. Some isolates of *B. ovis* may not grow readily on certain media, and the use of more than one type of medium is often recommended. Some commercial bacterial identification systems can misidentify *Brucella* as another organism. Treatment with antibiotics or bacterial overgrowth in nonsterile samples can interfere with culture.

B. ovis can be identified to the species level by phenotypic methods (phage typing and cultural, biochemical and serological characteristics) or genetic techniques. Diagnostic laboratories can identify most isolates of this organism; however, definitive identification may need to be done at a reference laboratory with experience in distinguishing brucellae. Most PCR tests only identify *Brucella* to the genus level, but a few *B. ovis*-specific PCRs have been published. Multiplex PCR assays that can distinguish several species of *Brucella* (e.g., the Bruce-ladder assay or the older AMOS test) are also used. Other tests that can be employed for species identification, such as single nucleotide polymorphism (SNP) typing and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), have been described. Techniques such as multiple-locus variable number tandem repeat analysis (MLVA) can aid epidemiological investigations of outbreaks. While PCR tests are often used to identify organisms in culture, some laboratories use these tests to identify *B. ovis* directly in clinical samples.

ELISAs, agar gel immunodiffusion (AGID) and complement fixation are usually used to detect antibodies to *B. ovis* in sheep, although other tests (e.g., hemagglutination inhibition, indirect agglutination) have been published. Rams with ambiguous tests should be isolated and retested after 2-4 weeks. *Dichelobacter nodosus*, which causes foot rot, was reported to cross-react with *B. ovis* in serological assays, but the practical significance is unknown. *B. ovis* has "rough" lipopolysaccharide in its cell wall, and serological tests for this organism do not cross-react significantly with *B. melitensis* or other *Brucella* species that contain "smooth" LPS.

Treatment

Some valuable rams have been treated successfully with antibiotics; however, fertility may remain low even if the organism is eliminated, and treatment is not economically feasible for most animals. It is advisable to keep treated rams separate and to culture their semen to ensure that organisms are no longer being shed.

Control

Disease reporting

Veterinarians who encounter or suspect brucellosis should follow their national and/or local guidelines for disease reporting. State authorities should be consulted for the reporting requirements in the U.S., where *B. ovis* is endemic.

Prevention

B. ovis is generally introduced into a flock in infected animals or semen. Flocks free of this organism should not be allowed to contact sheep that may be infected. Rams should especially not be housed with rams of unknown *B. ovis* status, or allowed to breed ewes that were recently mated by such rams. Rams can also transmit *B. ovis* to red deer, and possibly to other susceptible cervids or small ruminants, if they are kept in the same enclosure. In some countries, sheep are tested for *B. ovis* before entering artificial insemination facilities, or before movement across national or state borders. *B.-ovis*-free accredited flocks and rams may sometimes be available. Other rams should be tested before adding them to an uninfected flock.

Flocks can be screened for *B ovis* by palpating the scrotum of all rams, followed by laboratory tests on animals with abnormalities and some of the clinically normal rams. Ultrasound may aid in the clinical assessment. Because a significant number of infected rams do not have detectable lesions, the efficacy of palpation for screening can be influenced by the number of rams in a flock. If ovine epididymitis is diagnosed, all of the rams can be culled, or a test-and-slaughter program can be used to eliminate only the infected rams. Separating the rams on a farm into small

groups may help limit transmission until eradication is complete. Infections in ewes are generally prevented by controlling *B. ovis* in rams.

If eradication is not feasible, rams may be examined before the breeding season and any animals with palpable abnormalities culled. However, this only removes rams as they become clinically affected, and is less desirable than eliminating the organism from the flock. Vaccination can also help control ovine epididymitis, especially where eradication would be difficult. The *B. melitensis* Rev-1 vaccine is generally used; however, it interferes with surveillance for *B. melitensis*, and vaccination may not be allowed in some countries. A commercial killed *B. ovis* vaccine was employed at one time in New Zealand, but it was withdrawn due to incomplete immunity and injection site reactions.

Morbidity and Mortality

The flock prevalence of *B. ovis* is reported to range from <10% to nearly 50% in major sheep-raising regions. Although infections have been reported in rams as young as 4-6 months, they are more common in older rams. Whether goats can play any role in the epidemiology of this disease is uncertain. In Brazil, goats were more likely to be seropositive if they came from mixed sheep-goat herds, suggesting that infected sheep were the major source of exposure. The incidence of ovine epididymitis may be increasing in some countries where Rev-1 vaccination for *B. melitensis* is no longer practiced, such as France.

The effects of *B. ovis* vary between rams: this organism has little effect on sperm quality in some animals, but causes severe decreases in sperm motility, concentration and morphology in others. Male red deer seem to be less susceptible to *B. ovis* than sheep, with milder lesions and shorter carriage. Clinical cases also seem to be milder in experimentally infected male (billy) goats. However, experimentally infected bighorn sheep appeared to be at least as susceptible as domesticated sheep, with lesions that were similar or more severe. Estimates of the abortion rate and perinatal mortality caused by *B. ovis* vary, with some sources reporting rates of 1-2%, and others suggesting that these outcomes are rare. Up to 50% of ewes either aborted or gave birth to weak or stillborn lambs in some experiments; however, these ewes were inoculated intravenously at a susceptible stage of gestation, and are not thought to be representative of naturally infected flocks.

Internet Resources

Public Health Agency of Canada. Material Safety Data Sheets
https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment.html

The Merck Veterinary Manual
http://www.merckvetmanual.com/

World Organization for Animal Health (OIE)
http://www.oie.int

OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals
http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/

OIE Terrestrial Animal Health Code
http://www.oie.int/international-standard-setting/terrestrial-code/access-online/

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